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IMAGE ANALYSIS PROGRAM FOR MEASURING PARTICLES WITH THE ZEISS CSM 950 SCANNING ELECTRON MICROSCOPE (SEM)

BY

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JANUARY 1990

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A menu-type computer program for stereological particle measurement was written for use with a Zeiss CSM 950 Scanning Electron Microscope (SEM) having a built-in Kontron image analyzer. The program enables the user to perform a variety of parameter measurements from external sources such as a light microscope or video camera.					
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SUMMARY

The summary of each step in the image analysis program for measuring particles using the Zeiss CSM 950/Kontron system is as follows:

A>CSM calls the image analysis program.

Press D to define the program. Then, after pressing Return, type in 006 or 007.

Once the menu appears, the sequence of steps can be advanced by lighting up STEP at the top of the menu by using the mouse.

1. SCALE is set to 1 (SCNO 1). When using the microscope, 1 may have to be changed.
2. RESET. Allows you to go through the program from the beginning.
3. AREA. Highlight HIGH with mouse, type in a new maximum number, then Return.
4. FERET. Highlight HIGH with mouse, type in a new maximum number, then Return.
5. PERIM. HIGH and IOLT have to be same number, based on HIGH. Highlight first HIGH, then IOLT with mouse, type in a new maximum number, then Return.
6. DCIRCL is the diameter of circle being measured. Highlight HIGH with mouse, type in a new maximum number, then Return.
7. LAB stands for label.
8. TVON. The image can be moved.
9. SYNC. The image can be moved.
10. PAUSE. The program will pause here.
11. TVINP. The TV input will happen here.
12. DISC2L. The image is being discriminated from the background.
13. MFRAME. A frame is created.
14. IDENT. Unwanted images are eliminated.
15. MEAS. Selected parameters are measured. Particles touching the frame's edges are removed. When the mouse is hit again you stay in MEAS mode but now the X and Y block at the lower right corner of the menu screen lights up. Use the mouse to move the X to the block's lower left, then to the upper left. Now return to fix the coordinates. Be sure to write down what the coordinates are, e.g., X=15, Y=15.
16. OUTCLS. Histograms appear. Press the left arrow key to get next histogram. Hit P if you want to print a histogram. Be sure to write down MAX value so that the X axis value can be changed.
If the MAX is a value that is too small (or large) wait until the end of the program to return to the appropriate menu, i.e., if the X axis is 50 and the MAX reading on the histogram is 95, the 50 should be changed to 100.
17. OUTSGL. Data tables are presented. Only 19 values per screen are presented. Press down arrow key to go to next data screen if there are more than 19 values. Hit P to print 1st screen and each succeeding screen.
18. PAUSE. Last step in program.

PREFACE

This report describes the procedures we followed to write a menu-type program for performing stereological measurements of particles. By carrying out a dialogue with the screen menu, we were able to perform a step-by-step analysis of the current sequences of the program. What made this process especially difficult was the fact that the Kontron instruction manual defied understanding. It took 12 weeks to put the program together with the Kontron manual as our sole printed reference (June through August 1989).

Thanks are due to Dr. Nick Mace and Mr. David Bell of Cabot Corporation, Billerica, Massachusetts for their help with deciphering the manual and for their many useful suggestions.

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GLOSSARY OF TERMS

AREA.	The specific area of an object being measured.
AUX.	Auxiliary image memory.
BINARY.	A binary image is produced.
CALIBRATE.	The group which contains functions for set up and calibration of the image analysis program.
CHAN.	Video channel selection for digitization of image.
CLASS.	Process parameters for one or more measurement groups.
8-CONN.	Turns on image identification memory.
DCIRCL.	Measurement of the diameter of an area-equivalent circle.
DISC2L.	The function which separates objects from the background.
EVALUATE.	The group which includes functions for identification and measuring image objects and for controlling data output.
FERET.	Measurement of the diameter of an object in the X and Y directions.
FLG#.	Logic flag indicating buffer overflow.
HALT.	Data is displayed on monitor.
HIST.	Measured data in histogram form.
IDENT.	The function which identifies discriminated images and eliminates objects such as those touching the edge of the screen.
INPUT.	This function group controls the input of images into the system from external devices (SEM, TV-camera, diskettes, etc.).
INP.	Image memory containing the image to be processed.
LAB:.	A marker in the range of -999 to 999 in the sequence of a measuring program.

LBL#. Identifier of the label to which a jump in the program is directed.

LEV1. Lower discrimination level for background.

LEV2. Upper discrimination level for background.

MARG. Controls the measuring frame as defined by MFRAME.

MDLX. Presentation and scaling of the x-axis in the histogram for the length of thinned linear structures.

MDLY. Presentation and scaling of the y-axis in the histogram for the length of thinned linear structures.

MDYX. Presentation and scaling of the x-axis of a histogram for FERET values.

MDYY. Presentation and scaling of the y-axis of a histogram for FERET values.

MEASUR. The function which executes the actual measurements of the selected measuring parameters.

MFRAME. Use of a rectangular or circular measuring frame to limit the finite size of the field of view.

MODX. Presentation and scaling of the x-axis in the histogram for all variables.

MODY. Presentation and scaling of the y-axis in the histogram for all variables.

NCLS. Number of classes when classification is selected for all variables.

NCLT. Number of classes when classification is selected for the length of thinned linear structures.

NCLY. Number of classes in the histogram for all variables when classification is selected for FERET values.

NFAC. Normalizes absolute frequencies.

OBJ. Object specific parameters are to be evaluated.

ONLINE. Switches the online status on or off.

OUT. Image memory into which the image is stored.

OUTCLS. This function displays the results of the classification in the form of a list and/or a histogram.

OUTSGL. This function displays the individual lists of all measured data and stores the data.

PARAMETERS. The function group which lists the parameters to be selected for measurement. Most parameters can be measured simultaneously.

PAUSE. This function interrupts the running of the measuring program.

PERIM. The measurement of the perimeter and/or length of an object.

RAD. Radius of a circular frame.

REJECT. Unwanted objects are deleted.

RESET. Before starting a new measuring sequence, some conditions such as data buffering, identification number, etc. can be reset.

SCALE. A unit of calibration for geometric measurements (e.g., mm is selected).

SCNO. Input of 2 points and their real distance apart. Or, the absolute scale factor if the appropriate magnification is known.

SEGMENT. Functions which transfer grey level images to multiphase or binary images.

SINGLE. Data buffer is cleared.

SPAC. Distance in pixels between consecutive lines of the line grid.

SYNC. The measurement program can be synchronized externally (e.g., by using a TV-camera) or internally (by using the SEM).

TVINP. This function stores a TV image in memory.

TVON. This function allows one to control the image sections and brightness of the camera image. The color monitor shows the image currently recorded by the camera. No storage takes place.

UTILITIES. This function group contains a series of useful auxiliary functions which, in part, can be activated by the status line on the menu (displayed on the monitor).

WDSX,WDSY. Left and lower sides of a rectangular frame.

XO,YO. Coordinates of the upper left portion of a rectangular frame.

AN IMAGE ANALYSIS PROGRAM FOR
MEASURING PARTICLES WITH THE
ZEISS CSM 950 SCANNING ELECTRON MICROSCOPE

INTRODUCTION

The objective of this report is to describe the steps taken to write an image analysis program for measuring the stereological features of particles using a Zeiss CSM 950 Scanning Electron Microscope (SEM) with a built-in Kontron Image Analysis System.

The CSM 950 utilizes a CP/M operating system having a Z80 processor with a 20 Mbyte hard disk, 64 Kbyte system Random Access Memory (RAM) and 16 Kbyte video RAM.

Programs for such an application are constructed so that there is direct interaction between a menu display and the keyboard (or a mouse). As a result, the parameters of the system can be changed depending upon the type of sample.

Because some of the research currently underway in these laboratories requires knowledge of particle size, shape, etc., a menu-based stereological program was written with this purpose in mind.

METHODS

Two types of samples were used. The first was a drawing of 29 spherical particles (8 were large, 21 were small) (Fig 1), which were visualized with a Dage MTI Model OCD 72S video camera with a Fuji C6 x 17.5B TV zoom lens.

The second sample consisted of carbon particles (Fig 2) dusted onto the surface of a glass slide and examined with a Zeiss Ultraphot Camera Microscope using a 10x Planapo objective lens. The image was visualized with a Panasonic Model WV-CD51/A OCTV camera.

Images from each TV camera were transferred to a Zeiss CSM 950 Scanning Electron Microscope with a built-in Kontron image analysis system (Anonymous, 1986).

The CSM 950 SEM has a built-in Kontron image processing system, which, in general, performs evaluations in a series of steps: 1. input, digitization and storage of image from a TV camera, SEM, light microscope, etc.; 2. process grey image for improved contrast and signal/noise ratio; 3. extract features by global or local contrast; 4. analyze features (parameters such as shape, size, etc.); 5. output data for statistical processing.

FUNCTIONS

There are several major functions, which are arranged in groups relating to the specific sequence of a standard measuring procedure. These functions are: 1. Input - defines type of input device and controls image digitization and storage.

2. Calibrate - controls scaling factors.

3. Enhance - rescales the grey levels and enhances image quality with convolution filters.

4. Image Edit - enables the evaluation of image objects interactively.

5. Segment - extracts image background.

6. Multiphase - enables further processing of binary and multiphase images.

7. Parameters - permits the selection of object-specific, field-specific, and densitometric measuring parameters.

8. Evaluate - evaluates objects and outputs the results.

9. Advanced - performs geometric and arithmetical transformation of images.

10. Utilities - has general auxiliary functions.

11. Peripheral - permits control of peripheral devices, remote control, etc.

The above functions all have subfunctions that are displayed on the video monitor in the form of a menu, which displays the system's present mode. The use of dialogue with the program, allowing the operator to assign numerical values or delete them, as well as control of all system functions takes place by utilization of the keyboard or a mouse. What follows is the sequence for the operator to select to obtain the desired program.

OPERATOR SEQUENCE

A. Calibrate

1. Scale - As all measurement data are indicated in the selected unit of calibration, the most important prerequisite for every geometric measurement is calibrating the measuring system.

a. SCNO, the number of the scale factor to be activated, is set at 1.

B. Evaluate

1. Reset - Before starting a new measuring sequence, e.g., after loading a new program, some conditions such as data buffering, identification number and scanning stage can be reset. The system then has the same status as after initialization.

a. Single (on) - Clears the buffer into which the single data of several fields was accumulated.

b. Class (on) - Clears the sum-buffer for the total histogram (referring to all evaluated fields).

C. Select Parameter

1. Area - Object specific area.

a. NCLS (20) - Number of classes when classification is selected.

b. MODX (1-3) - Presentation and scaling mode of the x-axis in the histogram of the results.

c. MODY (1-10) - Presentation and scaling mode of the y-axis in the histogram of the results.

d. Single (on) - Selection of a single list.

e. Class (on) - Selection of a classification.

f. Low 0.000 - Lower bound of classification.

g. High 1300 - Upper bound of classification.

D. Select Parameter

1. Feret - Feret diameters in x and y direction.

a. NCLS (20) - Number of classes when classification (feature vectors) is selected.

b. MODX (1-3) - Presentation and scaling mode of the x-axis in the histogram of the results:

c. MDY (1-10) - Presentation and scaling mode of the y-axis in the histogram of the results:

d. Single (on) - Selection of a single list.

e. Class (on) - Selection of a classification.

f. Low (0.000) - Lower bound of classification.

g. High (50.00) - Higher bound of classification.

h. NCLY (20) - Number of classes when classification is selected.

i. MDYX (1-3) - Presentation and scaling mode of the x-axis in the histogram of the results:

j. MDYY (1-10) - Presentation and scaling mode of the y-axis in the histogram of the results:

k. LO.Y - Lower bound of the classification.

l. HI.Y - Upper bound of the classification.

E. Select Parameter

1. Perim - Object specific perimeter and length.

a. NCLS (20) - Number of classes when classification is selected.

b. MODX (1-3) - Presentation and scaling mode of the x-axis in the histogram of the results:

c. MODY (1-10) - Presentation and scaling mode of the y-axis in the histogram of the results:

d. Single (on) - Selection of a single list.

e. Class (on) - Selection of a classification.

f. Low (0.000) - Lower bound of classification.

g. High (150.0) - Upper bound of classification.

h. NCLT (10) - Number of classes when classification is selected.

i. MDLX (1-3) - Presentation and scaling mode of the x-axis in the histogram of the results:

j. MDLY (1-10) - Presentation and scaling mode of the y-axis in the histogram of the results:

k. LOLT (150.0) - Lower bound of classification.

l. HILT (0.000) - Upper bound of classification.

F. Select Parameter

1. DCIRCL - Diameter of area-equivalent circle.

a. NCLS (20) - Number of classes when classification is selected.

b. MODX (1-3) - Presentation and scaling mode of the x-axis in the histogram of the results:

c. MODY (1-10) - Presentation and scaling mode of the y-axis in the histogram of the results.

d. Single (on) - Selection of a single list.

e. Class (on) - Selection of a classification.

- f. Low (0.000) - Lower bound of classification.
- g. High (40.00) - Upper bound of classification.

G. Select Utilities

1. IAB: Label definition - Labels are used as markers in the sequence of a measuring program to which a jump can be executed.

- a. IBL#(1) - Identifier of the label, which serves as designation for a jump.

H. Input

- 1. TVON - Switches the color monitor directly to a TV signal.
 - a. Online (on) - Switches the online status on or off.

I. Select Utility

- 1. SYNC - The IPS can be synchronized externally (e.g., by using a TV camera) or internally. In the internal mode, no TV input is possible.
 - a. Intern (off), Internal (on), or External (off).

J. Select Utility

- 1. Pause - Interrupts run of program.

K. Input

- 1. TVINP - This function stores:
 - a. TV Input - A TV image in the memory indicated by INP
 - b. INP (1) - Image memory into which the image is to be stored.

L. Choose Segment

1. DISC2L - This function separates objects from the background, by setting two thresholds. Either the grey levels inside or those outside the entered limits are set to grey value 0 (black) and constitute the background.

Depending on the variable BINARY, the remaining object points either keep their original grey levels or are set to white (255).

- a. INP (1) - Image memory containing the image to be processed.
- b. OUT (2) - Image memory into which the discriminated image is stored.
- c. LEV1 - Lower discrimination limit.
- d. LEV2 - Upper discrimination limit.
- e. BINARY (on) - The object pixels become 255 (white), i.e. a binary image is produced.

M. Calibrate

1. MFRAME - This function is used to create a rectangular or circular measuring frame, which is sometimes necessary to statistically correct errors caused by the finite size of the field of view during the measurement process.

- a. INP (2) - Memory containing the image to be processed.
- b. WDSX (508) - X-side of the rectangular frame.
- c. WDSY (467) - Y-side of the rectangular frame.
- d. XO (4) - Position of the (rectangular or circular) frame.
- e. YO (6) - Position of the (rectangular or circular) frame.
- f. RAD (100) - Radius of the circular frame.

N. Evaluate

1. IDENT - This function serves two purposes: Identification of discriminated images and elimination of objects following the conditions set by the measuring frame (function MFRAME, group CALIBRATE).

- a. INP (2) - Image memory containing the image to be identified.
- b. OUT (3) - Memory containing the identified and frame corrected image.
- c. MARG (1) - Controls the meaning of the measuring frame defined by MFRAME (function group CALIBRATE).
- d. 8-CONN (on) - Method of identification, on = 8 CONN.

O. Evaluate

1. MEASURE - This function executes the measurement of the selected measuring parameters using PARAMETERS.

- a. OBJ - Must be switched on if object-specific parameters are among the previously selected parameters (AREA-TIME).
- b. INP (3) - Image memory containing the discriminated OBJ image.
- c. GRIM (1) - Image memory containing the original grey-image.
- d. AUX1 (9) - Auxiliary image memory.
- e. AUX2 (9) - Auxiliary image memory.
- f. CHAN (1) - Channel selection.
- g. SPEC (1) - Distance in pixels between consecutive lines of the "line grid".
- h. FLG# (1) - Logical flag indicating a buffer overflow.

i. REJECT (on) - Interactive rejection of undesired objects, preceding an automatic measurement.

P. Evaluate

1. OUTCLS - (Output of Classification) This function displays the results of the classification in the form of a list and/or as a histogram.

a. HISTO (on) - Provides measured data in histogram form.

b. HALT (on) - Presents data on the monitor; the sum histogram can be manipulated.

c. NFAC (0.000) - Defines the area (in user units) into which the absolute counts should be normalized. A value of 0.0 gives normalized absolute frequencies.

Q. Evaluate

1. OUTSGL - (Output of single list) This function displays the individual lists of all measured data.

a. HALT (on) - Displays lists on the data monitor after each measurement.

R. Select Utility

1. Pause - This function interrupts the running of a measuring program.

RESULTS

The first program (Table 1) was used to measure the Area, Perimeter, Feret X and DCircle of 29 circles (8 large and 21 small) so that the number and approximate size was known. The plots for each of the parameters (Figs. 3-6) showed an expected bimodal configuration. The printout of the statistical data can be seen in Table 2.

The second program (Table 3) measured a random number of carbon particles. The inputs to the parameters of this program (Area, Feret X and DCircle) were changed slightly from those of the first program to take into consideration the unknown number of particles seen in the visual field.

The Area (high upper bound of classification) was changed to 3500; the Feret X (high upper bound of classification) was changed to 300; and the DCircle (high upper bound of classification) was changed to 70. These changes were made to measure accurately the stereological parameters of different sized carbon particles. The plots for each parameter can be seen in Figs. 7-10 and the printout of the statistical data can be seen in Table 4.

CONCLUSIONS

Programs were written to allow for stereological measurement of carbon particles and subsequent statistical evaluation. The instrument used was a Zeiss CSM 950 SEM with a built-in Kontron image analysis system and the program was a menu type, which permitted direct interaction between the video monitor and the keyboard.

REFERENCES

Anonymous. 1986. Kontron SEM-IPS Operators Manual Volume II, Release 4.4, 410 pages.

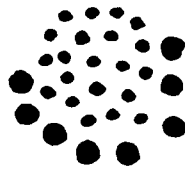


Figure 1.

Outline of 8 large and 21 smaller spherical particles to be measured.

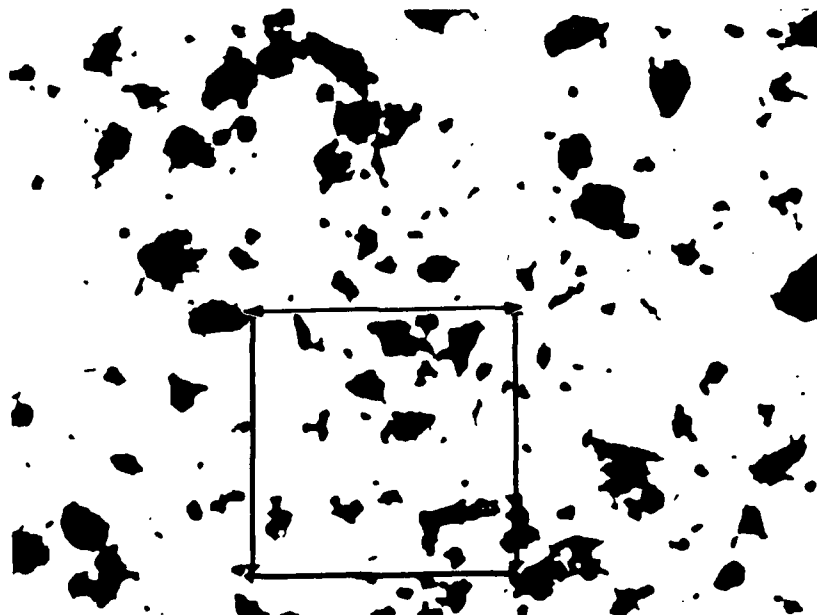


Figure 2.

Visual field of carbon particles as seen using light microscope. Within the outlined square are the particles actually measured.

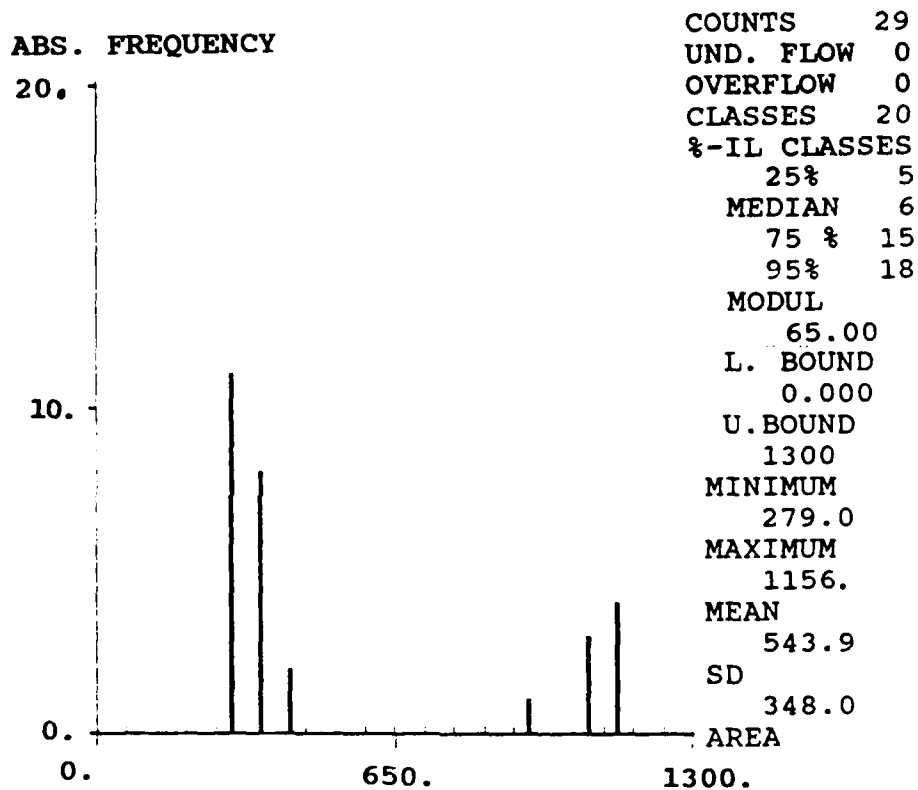


Figure 3. Histogram of area measurements from Fig. 1. The x axis values are in pixels. The y axis represents the frequency distribution.

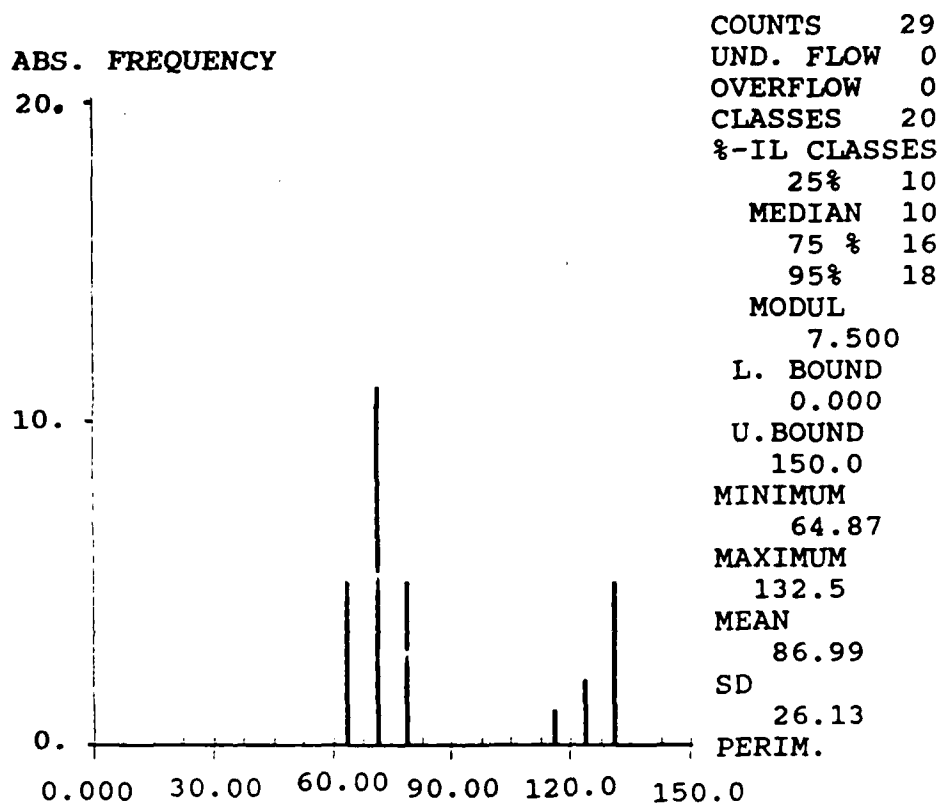


Figure 4. Histogram of perimeter measurements from Fig. 1. The x axis values are in pixels. The y axis represents the frequency distribution.

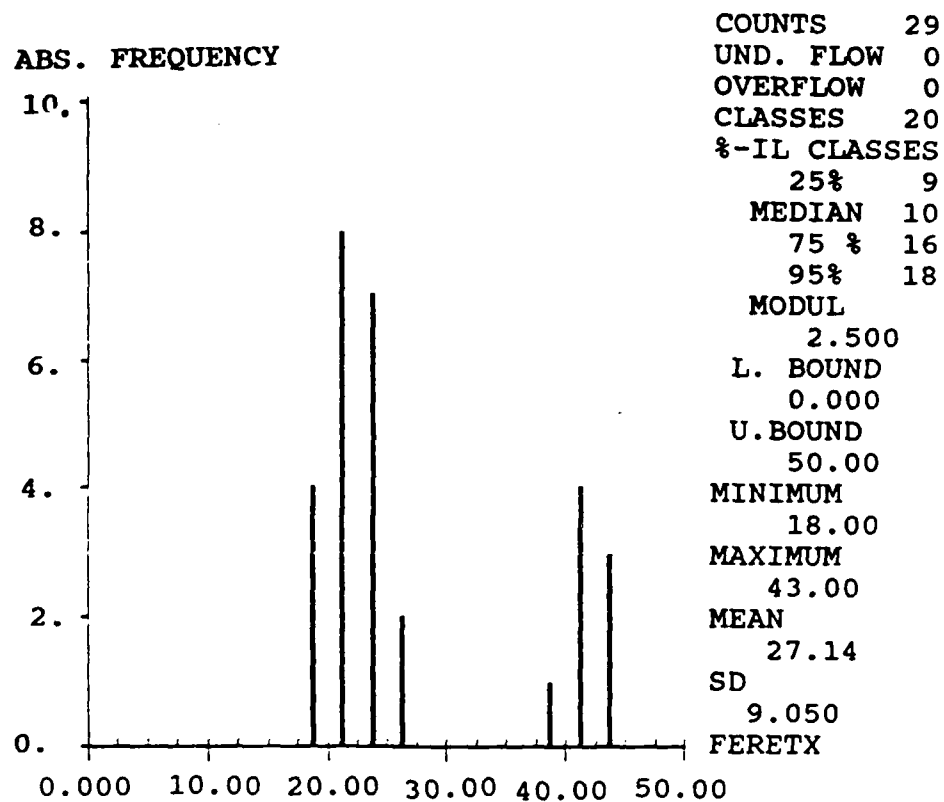


Figure 5. Histogram of Feret X measurements from Fig. 1. The x axis values are in pixels. The y axis represents the frequency distribution.

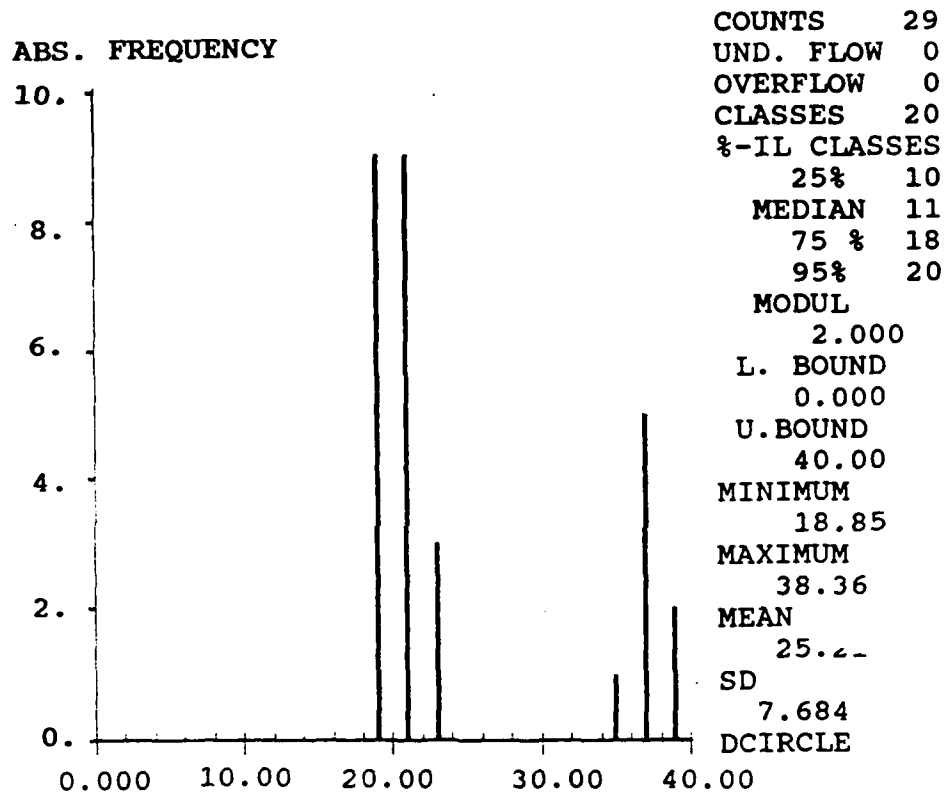


Figure 6. Histogram of an area equivalent to a circle (called DCircle) within Fig. 1. The x axis values are in pixels. The y axis represents the frequency distribution.

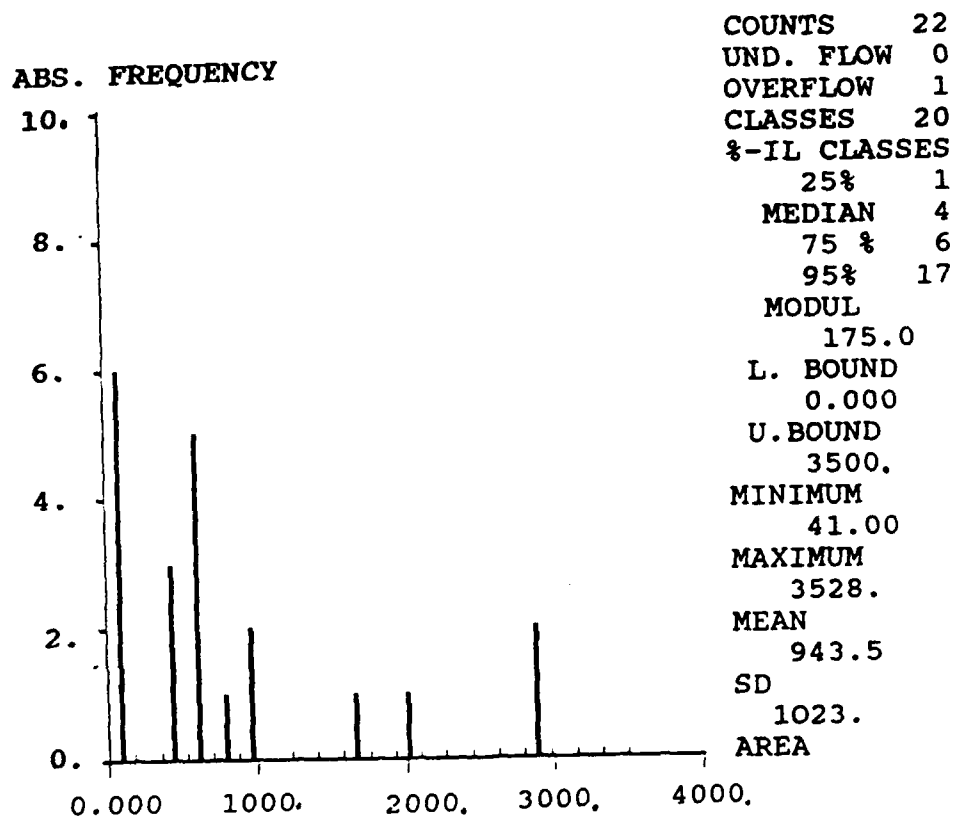


Figure 7. Histogram of area measurements from Fig. 2. The x axis values are in pixels. The y axis represents the frequency distribution.

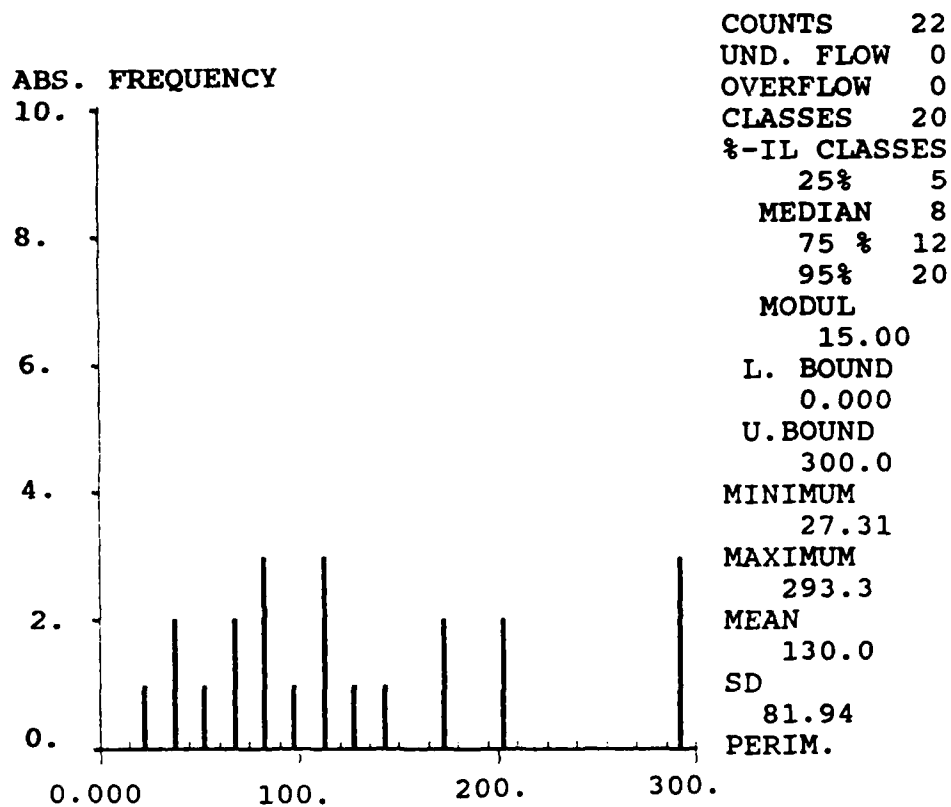


Figure 8. Histogram of perimeter measurements from Fig. 2. The x axis values are in pixels. The y axis represents the frequency distribution.

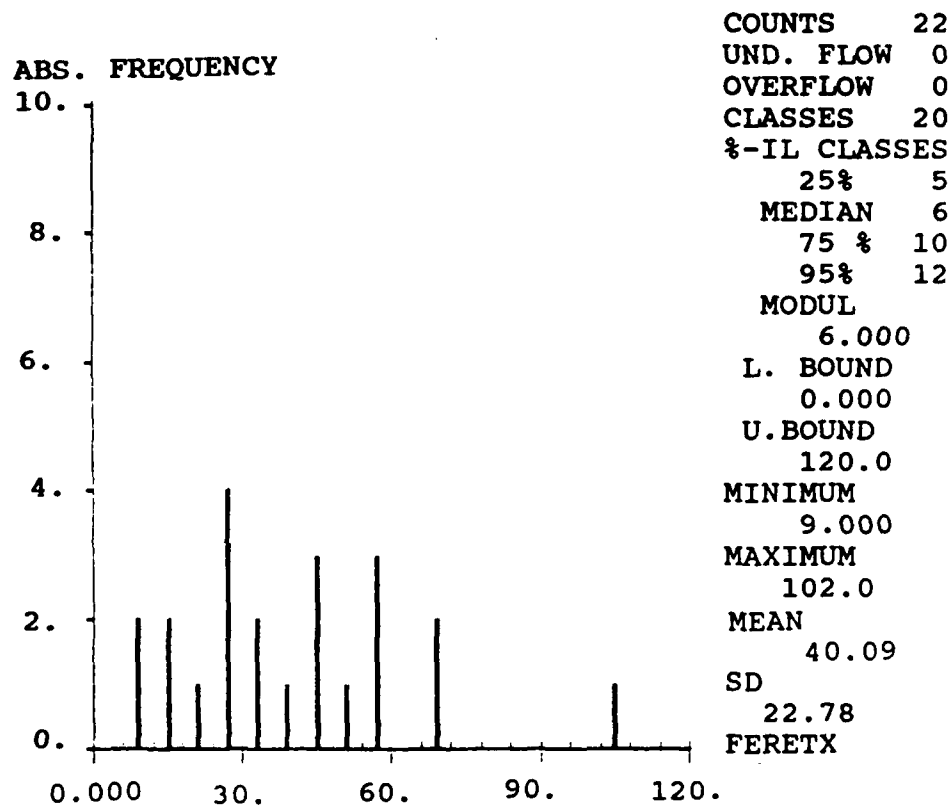


Figure 9. Histogram of Feret X measurements from Fig. 2. The x axis values are in pixels. The y axis represents the frequency distribution.

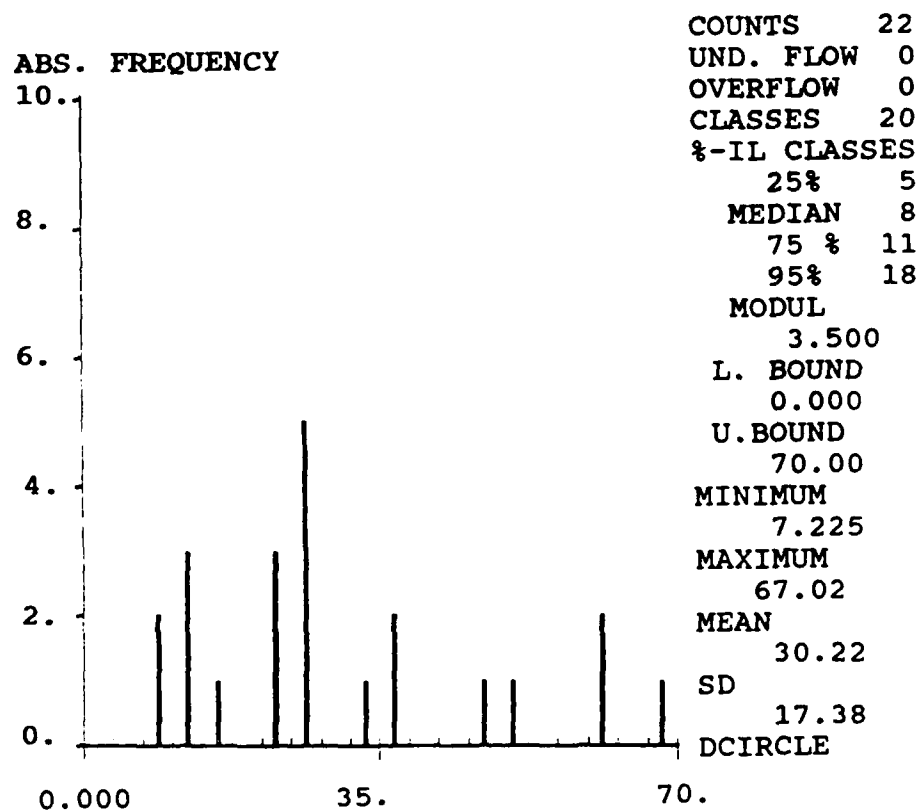


Figure 10. Histogram of an area equivalent to a circle (called DCircle) within Fig. 2. The x axis values are in pixels. The y axis represents the frequency distribution.

TABLE 1. Program for measuring 29 particles using the Kontron Image Analyzer.

MAJOR FUNCTIONS	SUB FUNCTIONS	INTEGER VALUES	VARIABLES	SCALE VALUES
A. CALIBRATE	SCALE	SCNO 1		
B. EVALUATE	RESET			
			SINGLE CLASS	
			PARAM	
C. PARAMETERS	AREA	NCLS 20 MODX 1 MODY 1	SINGLE CLASS	LOW 0.000 HIGH 1300.
D. PARAMETERS	FERET	NCLS 20 MODX 1 MODY 1 NCLY 20 MDYX 1 MDYY 1	SINGLE CLASS	LOW 0.000 HIGH 50.00 LO.Y 0.000 HI.Y 1000.0
E. PARAMETERS	PERIM	NCLS 20 MODX 1 MODY 1 NCLT 10 MDLX 1 MDLY 1	SINGLE CLASS	LOW 0.000 HIGH 150.0 LOLT 150.0 LILT 0.000
F. PARAMETERS	DCIRCL	NCLS 20 MODX 1 MODY 1	SINGLE CLASS	LOW 0.000 HIGH 40.00
G. UTILITIES	LABI	LBL# 1		
H. INPUT	TVON	ONLINE		
I. UTILITIES	SYNC			

TABLE 1. (Continued)

MAJOR FUNCTIONS	SUB FUNCTIONS	INTEGER VALUES	VARIABLES	SCALE VALUES
J. UTILITIES	PAUSE			
K. INPUT	TVINP	INP 1		
L. SEGMENT	DISC2L	INP 1 OUT 2 LEV1 0 LEV2 118	BINARY	
M. CALIBRATE	MFRAME	INP 2 WDSX 508 WDSY 467 XO 4 YO 6 RAD 100		
N. EVALUATE	IDENT	INP 2 OUT 3 MARG 1	8-CONN	
O. EVALUATE	MEASUR	INP 3 GRIM 1 AUX1 9 AUX2 9 CHAN 1 SPAC 1 FLG# 1	OBJ REJECT	
P. EVALUATE	OUTCLS		HIST	NFAC 0.000
			HALT	
Q. EVALUATE	OUTSGL		HALT	
R. UTILITIES	PAUSE			
TOTAL PROGRAM LENGTH		340 BYTES		

TABLE 2. Statistical data (in pixels) for 29 particles measured.

<u>COUNT</u>	<u>AREA</u>	<u>PERIM.</u>	<u>FERET X</u>	<u>DCIRCLE</u>
1	1156.0	132.0	43.0	38.36
2	1128.0	132.5	43.0	37.96
3	974.0	120.0	39.0	35.22
4	343.0	71.9	23.0	20.90
5	369.0	75.9	23.0	21.68
6	300.0	69.3	21.0	19.54
7	350.0	74.8	24.0	21.11
8	1086.0	127.2	41.0	37.19
9	354.0	73.6	23.0	21.23
10	285.0	64.9	18.0	19.05
11	293.0	65.7	19.0	19.31
12	306.0	66.8	21.0	19.75
13	306.0	67.7	19.0	19.74
14	437.0	80.1	25.0	23.59
15	302.0	69.4	22.0	19.61
16	1141.0	128.8	43.0	38.12
17	315.0	68.8	21.0	20.02
18	379.0	77.01	23.0	21.97
19	416.0	79.36	25.0	23.01
20	279.0	65.9	19.0	18.85
21	309.0	66.5	21.0	19.84
22	314.0	69.4	21.0	19.99
23	329.0	73.36	23.0	20.47
24	1115.0	130.2	40.0	37.68
25	323.0	73.4	22.0	20.28
26	388.0	75.0	23.0	22.23
27	332.0	69.9	21.0	20.56
28	1059.0	125.5	40.0	36.72
29	1084.0	128.0	41.0	37.15
MEAN	543.90	86.99	27.14	25.21

TABLE 3. Program for measuring carbon particles using the Kontron Image Analyzer.

MAJOR FUNCTIONS	SUB FUNCTIONS	INTEGER VALUES	VARIABLES	SCALE VALUES
A. CALIBRATE	SCALE	SCNO 1		
B. EVALUATE	RESET			
		SINGLE CLASS		
		PARAM		
C. PARAMETERS	AREA	NCLS 20 MODX 1	SINGLE CLASS	LOW 0.000 HIGH 3500.0
D. PARAMETERS	FERET	NCLS 20 MODX 1 MODY 1 NCLY 20 MDYX 1 MDYY 1	SINGLE CLASS	LOW 0.000 HIGH 120.0 LO.Y 0.000 HI.Y 1000.0
E. PARAMETERS	PERIM	NCLS 20 MODX 1 MODY 1 NCLT 10 MDLX 1 MDLY 1	SINGLE CLASS	LOW 0.000 HIGH 300.0 LOLT 300.0 LILT 0.000
F. PARAMETERS	DCIRCL	NCLS 20 MODX 1 MODY 1	SINGLE CLASS	LOW 0.000 HIGH 70.00
G. UTILITIES	LAB:	LBL# 1		
H. INPUT	TVON		ONLINE	
I. UTILITIES	SYNC			

TABLE 3. (Continued)

MAJOR FUNCTIONS	SUB FUNCTIONS	INTEGER VALUES	VARIABLES	SCALE VALUES
J. UTILITIES	PAUSE			
K. INPUT	TVINP	INP 1		
L. SEGMENT	DISC2L	INP 1 OUT 2 LEV1 0 LEV2 118	BINARY	
M. CALIBRATE	MFRAME	INP 2 WDSX 508 WDSY 467 XO 4 YO 6 RAD 100		
N. EVALUATE	IDENT	INP 2 OUT 3	8-CONN	
O. EVALUATE	MEASUR	INP 3 GRIM 1 AUX1 9 AUX2 9 CHAN 1 SPAC 1 FLG# 1	OBJ REJECT	
P. EVALUATE	OUTCLS		HIST	NFAC 0.000
			HALT	
Q. EVALUATE	OUTSGL			
			HALT	
R. UTILITIES	PAUSE			
TOTAL PROGRAM LENGTH 340 BYTES				

TABLE 4. Statistical data (in pixels) or carbon particles measured.

<u>COUNT</u>	<u>AREA</u>	<u>PERIM.</u>	<u>FERETX</u>	<u>DCIRCLE</u>
1	1020.0	203.2	67.0	36.04
2	841.0	174.8	57.0	32.72
3	977.0	142.0	52.0	35.27
4	98.0	41.8	15.0	11.17
5	2082.0	199.8	60.0	51.49
6	82.0	40.1	11.0	10.22
7	3528.0	296.0	66.0	67.02
8	542.0	101.5	25.0	26.27
9	2941.0	276.1	60.0	61.19
10	465.0	87.5	28.0	24.33
11	561.0	127.8	45.0	26.73
12	608.0	106.3	34.0	27.82
13	1722.0	167.4	44.0	46.82
14	2864.0	292.1	102.0	60.39
15	41.0	25.9	9.0	7.23
16	136.0	72.9	31.0	13.16
17	408.0	83.0	29.0	22.79
18	173.0	61.2	21.0	14.84
19	372.0	81.0	27.0	21.76
20	134.0	45.8	15.0	13.06
21	599.0	114.8	41.0	27.62
22	<u>564.0</u>	<u>106.4</u>	<u>44.0</u>	<u>26.80</u>
MEAN	943.50	130.0	40.09	30.22